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Research Report

The effects of aging, housing and ibuprofen treatment on brain neurochemistry in a triple transgene Alzheimer's disease mouse model using magnetic resonance spectroscopy and imaging

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ABSTRACT

We investigated a triple transgene Alzheimer's disease (AD) mouse model that recapitulates many of the neurochemical, anatomic, pathologic and behavioral defects seen in human AD. We studied the mice as a function of age and brain region and investigated potential therapy with the non-steroidal anti-inflammatory drug ibuprofen. Magnetic resonance spectroscopy (MRS) showed alterations characteristic of AD (i.e. increased myo-inositol and decreased N-acetylaspartate (NAA)). Mice at 6 months of age showed an increase in myo-inositol in the hippocampus at a time when the $A\beta$ is intracellular, but not in amygdala or cortex. Myo-inositol increased as a function of age in the amygdala, cortex and striatum while NAA decreased only in the hippocampus and cortex at 17–23 months of age. Ibuprofen protected the increase of myo-inositol at six months of age in the hippocampus, but had no effect at 17–23 months of age (a time when $A\beta$ is extracellular). In vivo MRI and MRS showed that at 17–23 months of age there was a significant protective effect of ibuprofen on hippocampal volume and NAA loss. Together, these data show the following: the increase in myo-inositol occurs before the decrease in NAA in hippocampus but not cortex; the hippocampus shows earlier changes than does the amygdale or cortex consistent with earlier deposition of $A\beta$40–42 in the hippocampus and ibuprofen protects against multiple components of the AD pathology. These data also show a profound effect of housing on this particular mouse model.

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1. Introduction

The cost of Alzheimer's disease (AD), both in human and financial terms, is expected to place an increasing, essentially unsustainable, burden on healthcare systems worldwide due to aging populations. It is crucial to find potential therapies that can either prevent the disease or slow progression. Epidemiologic studies of patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) for at least 24 months showed a large decreased subsequent relative risk of AD (in t' Veld et al., 2001; Stewart et al., 1997). Studies in Alzheimer's disease mouse models have shown protection against various aspects of behavioral and pathologic markers using NSAIDs (McGeer and McGeer, 2007) although clinical studies with patients who already have AD have been less successful (Jaturapatporn et al., 2012; Mullane and Williams, 2013). We showed using both magnetic resonance spectroscopy (MRS), as well as post-mortem histopathology that we could protect neuronal elements of the pathology in aged mice using treatment with ibuprofen in a double transgenic mouse model (PS1 × APP), although not all markers were responsive to the treatment (Choi et al., 2010b). This neuronal protection correlated with decreased *Aβ* plaque deposition. We also showed that treatment of triple transgene mice with ibuprofen was able to decrease not only the *Aβ* pathology but also hyper-phosphorylated tau (McKee et al., 2008). Magnetic resonance imaging and spectroscopy can provide non-invasive windows onto the neurodegenerative process and have become invaluable in assessing the impact of potential therapies in AD (Choi et al., 2007; Jack, 2012; Johnson et al., 2012; Westman et al., 2010).

MRS can provide information on both neuronal health and viability using the marker *N*-acetylaspartate (NAA) and also can provide information on glial markers such as myo-inositol a chemical that is elevated in both AD mouse models (Dedeoglu et al., 2004;(Choi et al., 2010b; Marjanska et al., 2005; Mlynarik et al., 2012; von Kienlin et al., 2005) as well as in humans (Kantarci et al., 2000; Pettegrew et al., 1997; Shonk et al., 1995). Using the decrease in NAA, coupled with the increase in myo-inositol as a simple ratio can sometimes provide better discrimination between treatment groups, or AD vs. controls than use of either metric alone (Choi et al., 2010b; Shonk et al., 1995). In addition to NAA and myo-inositol there are numerous other chemicals that can be measured including glutamate and glutamine, cholines, scyllo-inositol, GABA and taurine that can provide different windows onto the metabolic and pathological processes (Choi et al., 2007). In addition to the use of MRS, there have been great strides in the use of MRI for imaging various brain regions that show degeneration in AD such as hippocampus in both humans (Jack et al., 2012; Sabuncu et al., 2011) and in AD mouse models (Badea et al., 2010; Borg and Chereul, 2008; Redwine et al., 2003) that are correlated with disease progression. Therefore, in this study we used MRS (in vivo and in vitro) as well as MRI measurements of hippocampal volumes to asses the effects of aging and ibuprofen treatment in a triple transgene model of AD that manifests both *Aβ* and tau pathology (Oddo et al., 2003).

In addition to the effects of ibuprofen treatment, we also examined the effects of differential housing on the triple transgene animals. There is anecdotal evidence in the community (though not in the literature) that this particular AD model shows more variability than some other AD mouse models. There is good evidence that in a number of transgenic mouse models differences in housing can lead to different outcomes. Of particular relevance to AD was a paper that showed that anti-nerve growth factor mice raised in pathogen-free conditions, compared to conventional housing, had a significantly delayed onset of neurodegeneration (Capsoni et al., 2012). Thus, we present results here from mice housed in different facilities that demonstrate large differences between the facilities.

2. Results

We examined the effects of age on the AD temporal cortex using in vitro MRS. The data showed a trend for increased myo-inositol with increasing age that was not significant by linear regression ($R=0.34$; $p>0.1$) or ANOVA ($F_{2,21}=1.27$; $p<0.3$). There was also a decrease of NAA with age that was significant by linear regression ($R=0.438$; $p<0.05$) but not ANOVA ($F_{2,21}=2.36$; $p<0.1$), that is largely driven by the oldest ages. Using the metric myo-inositol/NAA provides additional power to detect changes (linear regression $R=0.66$; $p<0.001$; $F_{2,21}=7.35$; $p<0.01$). The increase in effect size, *r*, calculated from Cohen's *d* was 0.305, −0.267 to 0.528 for myo-inositol, NAA and myo/NAA, respectively. No changes in the creatine concentration were noted as a function of age ($R=0.18$; $p>0.4$; $F_{2,21}=0.77$; $p>0.45$). There was also a trend for an increase in the total choline concentration ($R=0.463$; $p<0.05$; $F_{2,21}=2.66$; $p<0.1$). These data are shown in Fig. 2.

We switched to HRMAS for the hippocampus and amygdala as it allows for smaller tissue punches to be studied due to the difficulties in the extraction of tiny tissue samples, and the amygdala is quite small. We also examined the caudate/putamen as a control region where pathology is not anticipated using 1 mm punches from the different brain regions as shown in Fig. 1. At six months of age the biggest changes were noted in hippocampus. There was a significant increase

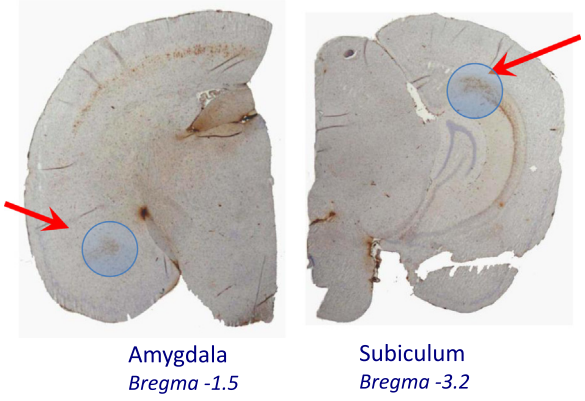

Fig. 1 – Picture of a post-mortem 3 × Tg brain with blue circles indicating the punches from amydala and subiculum area of the hippocampus. The brain is stained with 6E10 antibody showing the pattern of accumulation in the brain.